

THE DEVELOPMENT OF THE OVULE AND THE SEED IN THE HYPOXIDEAE.

PART II.

THE GENERA *PAURIDIA* HARV. AND *FORBESIA* ECKLON.

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Since the systematic position of the monotypic genus *Pauridia* still seems to be rather uncertain, the development of the ovule and the seed of *P. minuta* was investigated to determine whether this would throw any new light on the position of the genus. *P. minuta* resembles members of the genus *Ianthe*, especially *I. minuta*, but has only three stamens. Bentham and Hooker tentatively placed the genus under the Hypoxideae; Hutchinson, however, places it in the Hypoxidaceae, but Pax (Engler and Prantl) considers it to belong to the Haemodoraceae.

***Pauridia minuta* (L.f.) Dur. et Schinz.**

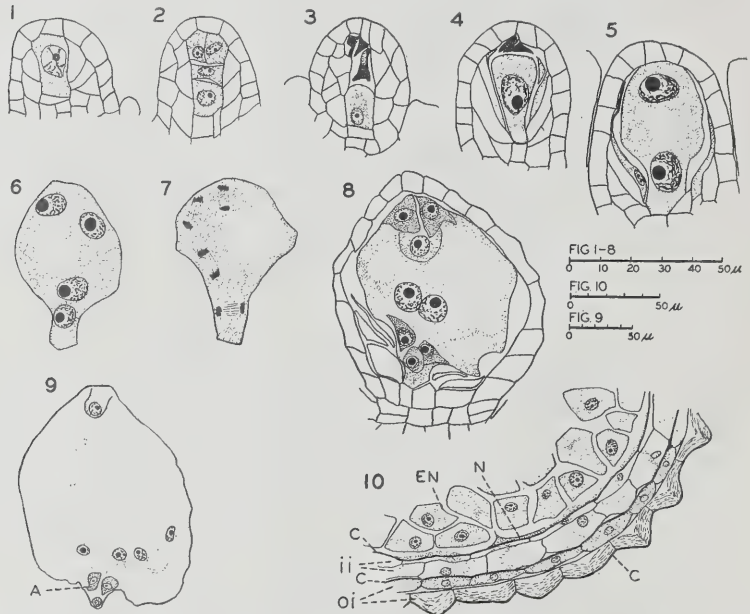
As in the genus *Ianthe*, the ovary is trilocular with axile placentation. Markötter (1936) considered the ovules to be anatropous. Usually, however, the raphe is short and does not reach the region of the micropyle. Such ovules should rather be called hemitropous. In a few cases the raphe is longer and the ovule therefore truly anatropous.

Gametogenesis. The archesporial cell, which is hypodermal in origin, functions directly as a megaspore mother-cell and enlarges considerably before the reduction division. After the first reduction division a cell-wall is laid down between the two daughter nuclei, and after the second division four megaspores are formed, usually in a T-shaped arrangement, but in a few cases in linear position.

While the three upper megaspores degenerate and disappear, the chalazal one enlarges and divides three times to form a normal 8-nucleate embryo-sac. The nucellus is small and consists of a number of cells at the chalaza and an epidermis covering the embryo-sac. Both integuments take part in the formation of the micropylar canal, and each consists of only two layers of cells.

The mature embryo-sac which has a narrow base and wide upper part, contains the egg-cell and two synergids, three antipodal cells and the secondary nucleus, formed by the fusion of the two polar nuclei. The sac has a large vacuole and some peripheral cytoplasm in which starch grains are embedded. Starch is also present in the synergids and the antipodal cells but not in the egg.

Abnormalities frequently appear in some ovaries, *e.g.* the nucellus, containing a mature embryo-sac, may protrude from integuments too short to cover it; or the nucellus may be covered only by the outer integument, the inner one remaining short; or embryo-sacs of some ovules may be late in developing. After fertilization many ovules degenerate and this is probably due to the non-occurrence of fertilization.



FIGS. 1—10. *Pauridia minuta*, development of the embryo-sac and endosperm. 1. Hypodermal megaspore mother-cell. 2. Four megaspores. 3. Functioning megaspore, the others degenerating. 4. Chalazal megaspore enlarging. 5—7. Further development of the embryo-sac. 8. Young mature embryo-sac. 9. Early development of the endosperm. 10. Section through nearly ripe testa with a small part of the endosperm. A. Antipodals. C. Cuticle. EN. Endosperm. II. Inner Integument. N. Remains of Nucellus. OI. Outer integument. Figs. 1—8 $\times 480$; 9 $\times 200$; 10 $\times 280$.

The Endosperm. After the fertilization the embryo-sac enlarges considerably at the expense of some of the basal nucellar cells. By repeated nuclear divisions the primary endosperm nucleus produces a large number of nuclei embedded in a layer of cytoplasm along the periphery of the embryo-sac. Helobial development of the endosperm does not take place and no basal endosperm chamber is formed.

The antipodal cells persist for some time after the fertilization and in some cases one of them enlarges, eventually becoming binucleate.

Cell-walls are laid down between the endosperm nuclei after about 130 to 150 have been formed, when the young embryo is in the quadrant or the octant stage. At this stage the antipodal cells have nearly disappeared, their degeneration starting when the fertilized egg-cell begins to divide. By repeated cell divisions the endosperm later fills the embryo-sac completely with a thin-walled tissue containing large amounts of food reserves, found by Markötter to consist of protein and oil.

The Embryo. After a short resting stage the fertilized egg-cell, attached to the wall of the embryo-sac near the micropyle, begins to divide. The first division is transverse, thereby forming an apical and a basal cell, *ca* and *cb*. The following divisions take place in both cells : in the basal cell by a transverse wall, forming the cells *m* and *ci*, and in the apical cell by a longitudinal wall.

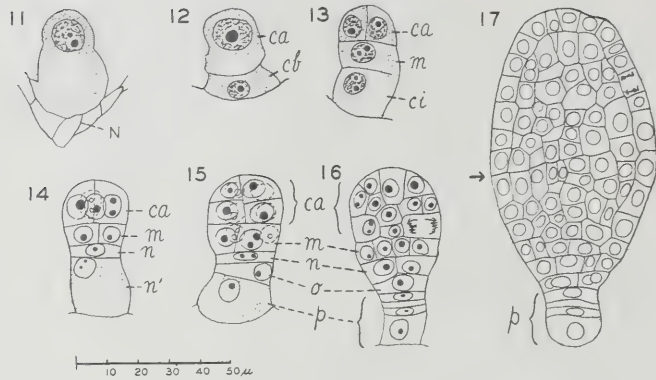
The octant stage is reached after another division in each of the four cells : in *ci* by a transverse wall forming the cells *n* and *n'*, and in *m* and in the two apical cells by longitudinal walls. Thus the original apical and basal cell each contribute four cells to the eight-celled embryo. In the next stage each cell divides again : the four upper cells divide by transverse walls so that two four-celled tiers are formed, the two middle cells by longitudinal walls at right angles to the first thus producing four cells, the cell *n* by a longitudinal wall, and *n'* by a transverse wall forming *o* and *p*.

After this 16-celled stage the divisions become increasingly irregular. The cells produced from the original apical cell form only the upper part of the embryo, *i.e.* the apical cotyledon. Cells produced from the layers *m*, *n*, and probably from *o*, all of which are derived from the basal cell *cb*, form the lower part of the embryo, *i.e.*, the radicle and hypocotyl. The cell *p* forms a short suspensor after undergoing a few further transverse divisions.

The embryo in the ripe seed is undifferentiated except for the dermatogen and a small dent on one side which shows the position of the growing point of the future plumule.

In *Pauridia* the development of the embryo is therefore identical with that of *Ianthe aquatica*. Both the original apical and basal cell contribute in large measure to the structure of the embryo, the development of which therefore conforms to the Asterad type (Johansen 1945).

The Testa of the seed is formed by both the integuments, each of which remains two-layered. The epidermal cells of the outer integument form rounded protuberances to the outside, but not as large as those in *Ianthe minuta*. The cavities of these cells become filled with cellulose



FIGS. 11—17. *Pauridia minuta*, development of the embryo. 11. Fertilized zygote. 12. Two-celled pro-embryo. 13. Quadrant stage. 14. Octant stage. 15. Embryo having nearly reached the sixteen-celled stage, cell *n* still having to divide longitudinally. 16. Beginning of the differentiation of the dermatogen. 17. Undifferentiated embryo of the nearly ripe seed. The arrow indicates the probable division between the original apical and basal cell. *ca*, apical cell; *cb*, basal cell; *N*, nucellus; *p*, suspensor; *m*, *n*, *o* and *p* develop from the original basal cell. $\times 480$.

laid down layer by layer within the outer cell-wall. Later this cellulose turns brown and eventually black due to the deposition of some other substance. The second cell layer of the outer integument is narrow, the cells retaining their protoplasmic contents until the seed is nearly ripe.

The outer layer of the inner integument consists of large thin-walled cells with very little protoplasm. They are, however, smaller than in the *Ianthe* species investigated and later they are compressed by pressure from the developing endosperm. The cells in the second layer are flat and contain a little protoplasm until a late stage.

Three narrow cuticular layers are present: the first on the outer surface of the seed-coat, the second between the two integuments, and the third between the nucellus and the inner integument. In the micro-pylar region the inner cuticle is folded as in species of *Ianthe*.

The nucellus at the top and along the sides of the embryo-sac is gradually absorbed, but at the base remnants of it are still visible in the nearly ripe seed.

***Forbesia plicata* (Ait.) Nel.**

As described by Markötter, the ovary has one chamber containing a number of ovules with long bent funicles, arranged in parietal placentation. They are hemitropous and at the same time slightly campylo-tropous, but not anatropous as stated by Markötter.

Gametogenesis. The hypodermal archesporial cell functions directly as megaspore mother-cell and undergoes meiosis. After the first reduction division a cross wall is laid down between the two interphasic nuclei. The second reduction division in the upper cell is incomplete, the cell contents degenerating before, during, or just after this division. The nucleus in the basal cell divides for the second meiotic division, but as no wall is laid down, this cell becomes binucleate. The nuclei are at first in close proximity and then move apart, a vacuole developing between them. They enlarge and undergo two further divisions to form the eight nuclei of the embryo-sac. The embryo-sac is therefore formed from two megasporic nuclei after two further mitoses.

When the flower opens the sac contains an egg-cell and two synergids, three very small antipodal cells and a definite nucleus formed by the fusion of the two polar nuclei.

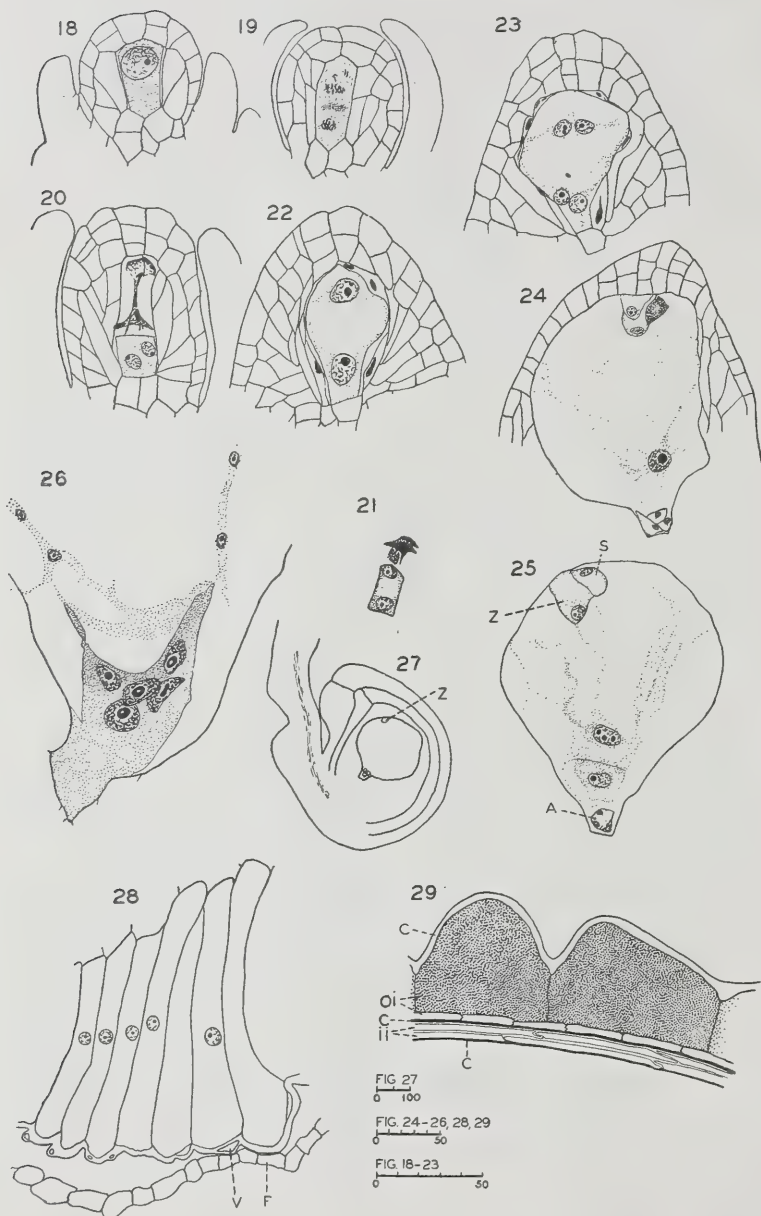
Before the reduction division the nucellar epidermis above the megaspore mother-cell divides and becomes two-layered, and while the embryo-sac develops these cells divide several times more, thus forming a large nucellar cap above the egg apparatus. On the sides the embryo-sac absorbs some of the nucellar cells, so that for a short distance the sac is covered only by the nucellar epidermis. The lower half of the embryo-sac, however, is embedded within a massive nucellar tissue, some of whose cell-walls in the immediate vicinity of the base of the sac show the presence of lignin.

The Endosperm. After the first division of the primary endosperm nucleus a cell-wall is laid down, cutting off one of the two nuclei in the basal end of the embryo-sac. This nucleus divides several times and at the same time the amount of cytoplasm in this basal chamber increases considerably. The nuclei increase in size and become hypertrophied, and the basal chamber forms a short protuberance into the nucellar tissue and probably functions as a chalazal haustorium. It does not however, touch the vascular strand which ends in the chalaza.

In the large upper chamber the endosperm proper develops. The nucleus divides repeatedly and forms a number of nuclei embedded in a layer of cytoplasm. Later cell-walls are laid down, thus forming a layer of cells, and by repeated cell divisions the embryo-sac becomes filled with endosperm tissue.

The antipodal cells are small and degenerate soon after the fertilization, their place being taken by the basal endosperm chamber.

The Embryo. The development of the embryo was observed only as far as the quadrant stage. The first division is transverse, and in the next divisions a longitudinal wall is formed in the apical cell and a transverse one in the basal cell, thus making the quadrant stage identical with



that of species of *Ianthe* and *Pauridia*. The following stages are probably similar to those in the above-mentioned plants. As in *Pauridia* and *Ianthe*, the embryo in the mature seed is undifferentiated.

The Testa. The integuments consist of two layers of cells except at their tips where they are thicker. The inner integument remains two-layered, the cells increase slightly in size and become very poor in protoplasmic contents. Later these cells are squashed and the cell walls are converted into an orange-coloured membrane which becomes either suberised or cutinised.

In the outer integument some of the cells of the inner layer divide by periclinal walls, making this integument subsequently for some part three-layered. The cells of the inner layer are small and possess large amounts of protoplasm which persist with their nuclei until the seed is nearly ripe, and some starch. The cells of the epidermal layer form protuberances to the outside and increase in size, especially those near the micropyle, where they elongate until they are six to nine times longer than broad. The ovule bends over in such a way that these palisade-like cells get into close contact with the funicle. Whether any nutritive substances can pass directly from the funicle into these integumentary cells, is impossible to say. The cuticle over these elongated cells shows curious vesicles (Fig. 28), but no direct break or pores were visible in it. Later the epidermal cells turn black and form the thick outer coat of the testa.

Three fairly thick layers of cutin are present in the seed-coat: the first on the outer surface, the second between the outer and the inner integument and the third on the inner surface of the latter.

The nucellus is absorbed except in the chalaza where some nucellar cells are still visible in the ripe seed. The rostelliform appendix at the hilum mentioned by Bentham and Hooker, is formed from the elongated epidermal cells of the outer integument near the micropyle, and from enlarged epidermal cells of the upper part of the funicle at the point where this is attached to the seed. The abscission of the seed takes place between these two groups of cells.

FIGS. 18—29. *Forbesia plicata*. 18. Megaspore mother-cell. 19. First reduction division of the megaspore mother-cell. 20. Functional binucleate megaspore cell, the upper one having degenerated. 21. Two nuclei of the functional cell moving apart. 22—23. Further development of the embryo-sac. 24. Mature embryo-sac after fertilization. 25. Two endosperm nuclei separated from each other by a membrane. 26. Multinuclear basal endosperm chamber forming a chalazal haustorium; above it some nuclei of the endosperm proper. 27. Longitudinal section through ovule showing the outer integument in contact with the funicle. 28. Elongated cells of the outer integument in contact with the funicle. 29. Seed-coat of the ripe seed. A. Antipodal cells. C. Cuticle. F. Funicle. II. Inner integument. OI. Outer integument. S. Synergidae. V. Vesicle in cuticle. Z. Zygote. Figs. 18—23 $\times 300$; 24—26, 28, 29 $\times 200$; 27 $\times 50$.

When the flower opens the ovary chamber becomes filled with pectin mucilage staining bright red with ruthenium. This mucilage later forms the succulent substance of the fleshy fruit and is probably excreted by the funicles and wall of the ovary, as these structures show large areas not covered by cuticle. In younger stages they possess intact cuticles, but the growth of the cuticle does not keep pace with the expansion of their epidermal surfaces. The funicles become broad and flat, a large cavity developing in them around the vascular strand.

DISCUSSION.

In *Pauridia minuta* the structure and the development of the ovule and seed are so greatly similar to those of *Ianthe* species that one can hardly believe that they belong to different genera. In both genera the development of the embryo-sac is normal and the embryo develops according to the Asterad type and in the ripe seed is still undifferentiated. The formation of the endosperm in *Pauridia* is similar to that of certain species of *Ianthe*, viz., *I. aquatica*, *I. alba* and *I. minuta*, and the seed-coat in the two genera is similar in structure. The only difference lies in the smaller size of the ovule and seed and also of certain cells and tissues in *Pauridia*, e.g., the nucellus and some cells of the testa.

This investigation confirms the view that *Pauridia* is very closely related to *Ianthe* (Hypoxis), and shows that Bentham and Hooker and Hutchinson were correct in placing *Pauridia* under the Hypoxideae as Bentham and Hooker tentatively did, or under the Hypoxidaceae as Hutchinson did. Brackett (1923) also states that the seeds of *Pauridia* are externally similar to those of Hypoxis, Curculigo and Molineria and that they should be classed together.

Too great stress should therefore not be laid on the presence of only one whorl of stamens in *Pauridia*, and the description of the Hypoxideae should be modified to include this genus. Pax (1930) placed the genus under the Haemodoraceae because of the absence of the outer whorl of stamens, but preliminary investigations in other members of the latter family show the development and structure of the ovule and seed to differ in certain respects from that of *Pauridia* and other members of the Hypoxideae.

In *Forbesia plicata*, another member of the Hypoxideae, the formation of the embryo-sac conforms to the Allium type (Maheshwari 1948), in which the 8-nucleate sac develops, after two further mitoses, from a binucleate cell regarded as the equivalent of two megasporos which have not separated. It therefore differs from the other members of the Hypoxideae investigated. Stenar (1925) found that even such a

closely related plant as *Curculigo recurvata* shows the normal type of embryo-sac development.

In *Forbesia plicata* the nucellus is much larger than in species of *Ianthe* and especially in *Pauridia*, where it is very small indeed.

The Helobial endosperm development which occurs in *Forbesia plicata* is similar to that described by Stenar (1925, 1938, p. 281) in two species of *Hypoxis* and by the present author in *Ianthe Schlechteri*. The basal endosperm chamber, however, is of larger size and may be considered a chalaza haustorium. Schlimbach (1924) also mentions the presence of a large chalazal haustorium in an unidentified species of *Curculigo*. Probably this also originated from a basal endosperm chamber.

There seems to be a connection between the persistence of the antipodal cells and the formation of Helobial endosperm. With large persistent antipodals nuclear endosperm development takes place and no basal endosperm chamber is formed. With small antipodal cells which degenerate early, Helobial endosperm development occurs, the basal endosperm chamber taking in the position of the antipodals. Which is cause and which effect is impossible to say.

Forbesia plicata is more specialized than either *Ianthe* or *Pauridia* species. This is shown not only in the structure of the flower (the presence of the long rostrum), but also of the ovary and ovule, *e.g.*, (i) the elongation of the epidermal cells of the outer integument to make contact with the funicle; (ii) the enlargement of the latter structure; (iii) the development of mucilage within the ovary and the subsequent development of a fleshy fruit, and (iv) if the normal type is taken as primitive, the *Allium* type of embryo-sac development.

SUMMARY.

1. In *Pauridia minuta* the archesporial cell is hypodermal and remains in that position, the embryo-sac being covered only by a nucellar epidermis.

2. The four megaspores formed after the reduction division are usually in a T-shaped position and the chalazal one is functional. The development of the embryo-sac is of the normal type and the endosperm is formed by nuclear development, the antipodals enlarging somewhat and persisting for some time after the fertilization.

3. The embryo develops according to the Asterad type.

4. The structure of the seed-coat is similar to that in the *Ianthe* species.

5. As the development of the ovule and seed of *P. minuta* is similar to that of the species of *Ianthe* investigated, it is concluded that these

two genera are very closely related and that they should be classed together.

6. In *Forbesia plicata* the archesporial cell is hypodermal, but later the nucellar epidermis at the top becomes many-layered and forms a large cap above the egg-apparatus.

7. The Allium type of embryo-sac development takes place, and Helobial endosperm formation occurs, a chalazal haustorium being formed from the basal endosperm chamber. The antipodal cells degenerate soon after fertilization.

8. The development and structure of the seed-coat is explained.

9. The structure of the ovary and the ovule confirms the view that *F. plicata* is more specialized than species of *Ianthe* and *Pauridia*.

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